

MANFRED T. REETZ ET AL.  
USSN 09/463,494  
RESPONSE TO FINAL REJECTION DATED APRIL 7, 2003  
AMENDMENT OF MARCH 8, 2004

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1.-41. (Canceled)

--42. (New)           A process for the preparation and identification of a mutant hydrolase having improved stereoselectivity or regioselectivity properties with respect to a substrate compared to a starting hydrolase from which said mutant hydrolase is derived, said method comprising the following steps:

- a)     providing a starting hydrolase gene encoding said starting hydrolase;
- b)     introducing one or more mutations into said starting hydrolase gene to produce a mutant hydrolase gene by subjecting said starting hydrolase gene to a mutagenic polymerase chain reaction (PCR), wherein said mutagenic polymerase chain reaction comprises adjusting one or more parameters of the reaction in order to control the number of mutations introduced into said starting hydrolase gene, wherein said one or more parameters are selected from the group consisting of the  $Mg^{2+}$  concentration of the reaction, the  $Mn^{2+}$  concentration of the reaction, the deoxynucleotide concentration of the reaction and the number of cycles of the reaction;

MANFRED T. REETZ ET AL.  
USSN 09/463,494  
RESPONSE TO FINAL REJECTION DATED APRIL 7, 2003  
AMENDMENT OF MARCH 8, 2004

- c) transforming a host organism with said mutant hydrolase gene, and expressing the mutant hydrolase gene to yield a mutant hydrolase; and
- d) testing and identifying a mutant hydrolase having improved stereoselectivity or regioselectivity properties with respect to a substrate compared to said starting hydrolase.--

--43. (New) The process according to claim 42, wherein a mutation rate of 1-2 base substitutions per starting hydrolase gene to be mutagenized in said PCR is established in step b) by adjusting one or more parameters of the reaction selected from the group consisting of the  $Mg^{2+}$  concentration of the reaction, the  $Mn^{2+}$  concentration of the reaction and the deoxynucleotide concentration of the reaction.--

--44. (New) The process according to claim 42, wherein the starting hydrolase gene is a hydrolase gene which has previously been mutagenized in a PCR previously performed in accordance with step b).--

--45. (New) A process for the preparation and identification of a mutant hydrolase having improved stereoselectivity or regioselectivity properties with respect to a substrate compared to a starting hydrolase from which said mutant hydrolase is derived, said method comprising the following steps:

MANFRED T. REETZ ET AL.  
USSN 09/463,494  
RESPONSE TO FINAL REJECTION DATED APRIL 7, 2003  
AMENDMENT OF MARCH 8, 2004

- a) providing a starting hydrolase gene encoding said starting hydrolase;
- b) introducing one or more mutations into said starting hydrolase gene to produce a mutant hydrolase gene by subjecting said starting hydrolase gene to a mutagenic polymerase chain reaction (PCR), wherein said mutagenic polymerase chain reaction comprises adjusting one or more parameters of the reaction in order to control the number of mutations introduced into said starting hydrolase gene, wherein said one or more parameters are selected from the group consisting of the  $Mg^{2+}$  concentration of the reaction, the  $Mn^{2+}$  concentration of the reaction, the deoxynucleotide concentration of the reaction and the number of cycles of the reaction;
- c) producing a recombinant hydrolase gene by enzymatically fragmenting a plurality of said mutant hydrolase genes or a mixture of at least one said starting hydrolase gene and at least one said mutant hydrolase gene to produce a plurality of gene fragments and recombining said gene fragments to yield at least one recombinant hydrolase gene having a nucleotide sequence different from either said mutant hydrolase genes or said starting hydrolase gene;
- d) transforming a host organism with said recombinant hydrolase gene, and expressing the recombinant hydrolase gene to yield a

MANFRED T. REETZ ET AL.  
USSN 09/463,494  
RESPONSE TO FINAL REJECTION DATED APRIL 7, 2003  
AMENDMENT OF MARCH 8, 2004

mutant hydrolase; and

- e) testing and identifying a mutant hydrolase having improved stereoselectivity or regioselectivity properties with respect to a substrate compared to said starting hydrolase.--

--46. (New) The process according to claim 45, wherein a mutation rate of 1-2 base substitutions per starting hydrolase gene to be mutagenized in said PCR is established in step b) by adjusting one or more parameters of the reaction selected from the group consisting of the  $Mg^{2+}$  concentration of the reaction, the  $Mn^{2+}$  concentration of the reaction and the deoxynucleotide concentration of the reaction.--

--47. (New) The process according to claim 45, wherein the starting hydrolase gene is a hydrolase gene which has previously been mutagenized in a PCR previously performed in accordance with step b).--